Original Article

Mixed exposure to As, Mn, and Pb and dopamine neurotransmission in the striatum

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ABSTRACT

Mixed exposure to arsenic (As), manganese (Mn), and lead (Pb) occur frequently in the general population; however, there are few studies on the neurotoxic effects, including those on dopaminergic neurotransmission in the striatum. This study investigated tyrosine hydroxylase (TH), dopamine transporter (DAT), and vesicular monoamine transporter 2 (VMAT2) expression in the mouse striatum after exposure to As, Mn, and Pb. Mixed exposure to these metals through drinking water for 4 weeks resulted in a significant reduction in the expression of TH and VMAT2 in the striatum of mouse, while the DAT expression was not significantly changed. These results suggest that the expression of TH and VMAT2 plays an important role in neurotoxicity caused by mixed exposure to As, Mn, and Pb. The results also indicate that synergy may occur with combined exposure to heavy metals such as As, Mn, and Pb, and is more likely to occur even when exposure of heavy metals is low.

Keywords: Neurotoxicity, Metal mixture, Dopamine synthesis, Striatum, Monoamine transporter

Introduction

The general public is exposed to a variety of metals, including arsenic (As), manganese (Mn), and lead (Pb), where exposure to these metals contributes to the disease and disability burden [1]. Exposure to high concentrations of As is known to be linked with various illnesses, including skin disorders, cancer, and cognitive impairment [2]. Numerous studies have reported that exposure to these metals is toxic to the central nervous system and that disturbances in dopaminergic neurotransmission are important to the development of neurotoxicity [3-6]. Dopamine regulates various aspects of brain function and is synthesized by tyrosine hydroxylase (TH) [7, 8]. During dopaminergic neurotransmission, extracellular dopamine secreted into the synaptic cleft is transported by the dopamine transporter (DAT). DAT, a plasma membrane protein selectively expressed in dopaminergic neurons, is critical to the regulation of dopaminergic neurotransmission [9]. Another major regulator of dopaminergic signaling is the vesicular monoamine transporter 2 (VMAT2). DAT and VMAT2 remove dopamine from the extracellular and cytosolic space, respectively, which determines the amount of dopamine present in the synaptic cleft [10]. Metals are known to accumulate in the basal ganglia, which mainly consists of the striatum, and exhibit neurotoxic effects through interactions with intracellular targets in dopaminergic neurons [11]. Therefore, this study aims to elucidate the effects of exposure to As, Mn, and Pb on the TH, DAT, and VMAT2 expression in the mice's striatum.

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Materials and Methods

Animals

C57BL/6 male mice (22.5 ± 0.5 g, 7-week-old) were used in the experiment. We divided mice into 5 groups: control, As (100 mg/L)-, Pb (100 mg/L)-, Mn (100 mg/L)-, and metal

mixture (50 mg/L As + 50 mg/L Mn + 50 mg/L Pb)-treated groups. The metal-treated animals (n = 5 per group) provided sodium arsenite, manganese (II) chloride tetrahydrate, and lead (II) acetate trihydrate, for 4 weeks. The control group was provided distilled water. Drinking water intake was measured during the entire experimental period and body weight was measured weekly. We use the highest quality products available for other reagents. The IACUC (Institutional Animal Care and Use Committee) of Keimyung University, Korea (approval No.: KM 2018-014) approved these experiments. Animal experiments were carried out following the NIH guidelines. After CO_2 asphyxiation, the mouse striatum was dissected from the brain.

Real-time PCR

For mRNA analysis, using the NucleoSpin RNA kit following the manufacturer's instructions, RNA was extracted. Then, using the iScript cDNA Synthesis, cDNA was synthesized. The SsoAdvanced Universal SYBR Green Supermix kit, supplied by Bio-Rad, is used in the characterization of cDNAs. The TH, DAT, VMAT2, and β-actin genes were analyzed. Data were evaluated based on the Cq number. To carry out Cq data analysis, CFX Manager (Bio-Rad) was used to determine the expression levels of genes.

Western blot analysis

To analyze the protein expression, the striatum of mice was homogenized and the homogenate was centrifuged at 14,000 rpm for 20 min at 4 °C. The protein level was assessed using the Bradford method. The standard used for the Bradford assay is bovine serum albumin (BSA). A 5 μg of protein was separated on 10% SDS-PAGE gels, and the proteins were transferred onto a nitrocellulose membrane. The TH, DAT, and VMAT2 primary antibodies were used in this study for immunoblotting. The immunoreactive signals were detected with enhanced chemiluminescence reagents and captured on X-ray film after incubation with secondary antibodies. The optical density of bands was analyzed using the NIH ImageJ (Bethesda, MD, USA).

Statistical analysis

Followed by a post hoc Duncan test, ANOVA was applied to compare the body weight, mRNA expression, and optical density of protein expression between metal-treated and control mice. A p values less than 0.05 or 0.01 were considered statistically significant. All statistical analyses were performed using SAS v. 9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The change in body weight of the experimental animals during the entire experimental period is shown in **Figure 1**. Compared with the control group, there was a slight reduction in the bodyweight of the group administered metals, although the change was not statistically significant.

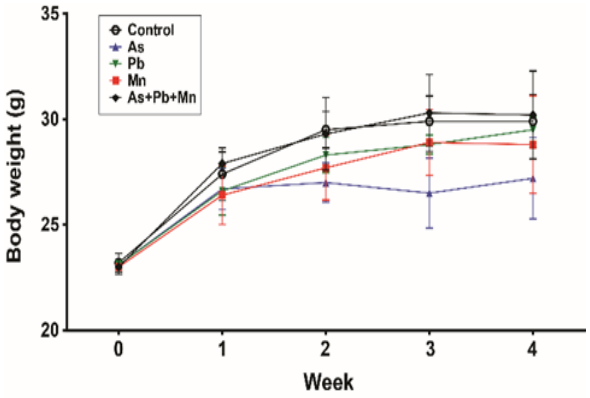

Figure 1. Change in body weight.

Table 1 summarizes the results of quantitative analysis of TH, DAT, and VMAT2 expression, which are important to dopaminergic neurotransmission, in the striatum of animals administered metals. TH expression decreased after the cut-off value (<0.5-fold) in all groups administered individual or mixed metals. DAT expression showed no significant change regardless of the metal administered. VMAT2 expression did not change significantly in the groups administered individual metals; however, a significant reduction in expression was observed in the group administered a metal mixture.

TH protein expression in the striatum of experimental animals is shown in **Figure 2**. Consistent with the analysis of TH mRNA, the TH protein expression was significantly decreased in groups administered individual or mixed metals ($p < 0.05$ or $p < 0.01$). These results are in accordance with the findings of previous studies showing that As, Pb, and Mn affected the expression of TH [12-14].

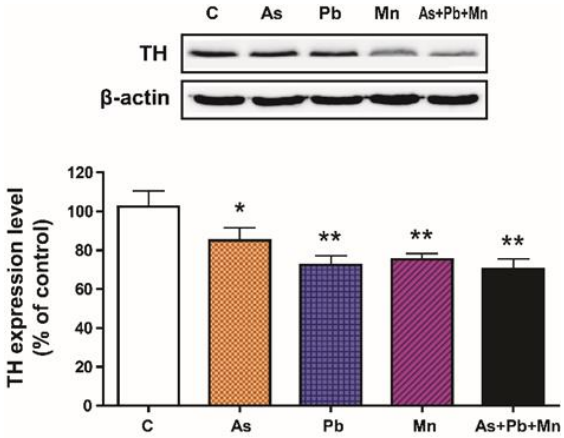

Figure 2. Protein expression of tyrosine hydroxylase (TH) treated with As, Pb, or Mn. Proteins detected by Western blotting (upper panal). Quantitative analysis of the relative expression of the protein (lower panal). *p<0.05, **p<0.01.

Like DAT gene expression, DAT protein expression did not change significantly with mixed or single metal treatment **(Figure 3)**. These findings suggest DAT may not have a direct

effect on the neurotoxicity induced by single or mixed exposure to As, Pb, and Mn.

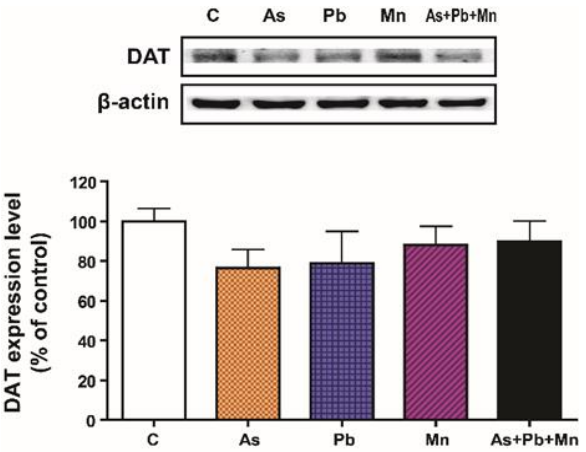

Figure 3. Protein expression of dopamine transporter (DAT) treated with As, Pb, or Mn. Proteins detected by Western blotting (upper panal). Quantitative analysis of the relative expression of the protein (lower panal).

VMAT2 protein expression did not change significantly with individual metal exposure, but a significant reduction in expression was observed in the mixed metal exposure group ($p < 0.01$). These results are consistent with the VMAT2 gene expression data **(Figure 4)**. While few studies have analyzed the effects of metal exposure on the expression of VMAT2, some have reported that As or Pb exposure reduces the expression of VMAT2 [12, 15]. In this study, mixed metal exposure significantly reduced the VMAT2 expression, but exposure to single metals did not. These discrepancies may be the result of differences in the dose and duration of the metal administered, as well as the brain regions analyzed.

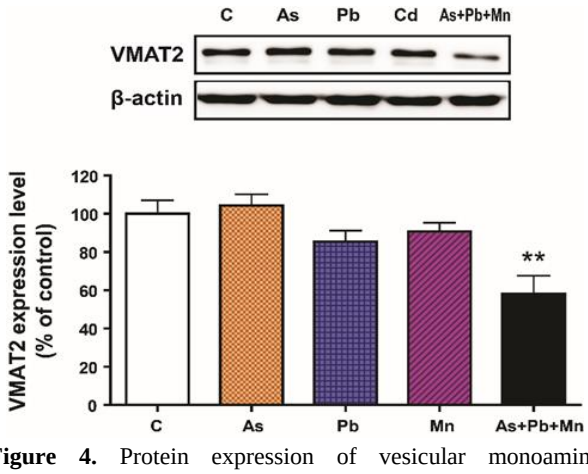

Figure 4. Protein expression of vesicular monoamine transporter 2 (VMAT2) treated with As, Pb, or Mn. Proteins detected by Western blotting (upper panal). Quantitative analysis of the relative expression of the protein (lower panal). * **p<0.01.

As, Pb and Mn often exist individually in the environment, but they can also be found as a mixture [16]. Interactions among metals may lead to toxicokinetic and toxicodynamic changes, resulting in enhanced toxicity [17, 18]. This toxic synergy can result when each metal acts through a similar mechanism in the same organ, leading to greater toxicological effects from metal mixtures.

Table 1. Quantitative analysis of gene expression in the striatum

Gene	NCBI RefSeq No.	Fold change[a]			
		As	Pb	Mn	As+Pb+Mn
TH	**NM_009377**	0.04±0.02*	0.36±0.41*	0.36±0.23*	0.11±0.03*
DAT	**NM_010020**	0.65±0.14	0.94±0.24	0.70±0.12	0.72±0.06
VMAT2	**NM_172523**	0.96±0.09	0.85±0.11	1.00±0.05	0.04±0.01*

[a] Gene expression was normalized based on the expression of β-actin. Fold changes relative to the control group were calculated. Values denote the mean ± standard error (SE) of each group (n = 3).

* Fold changes that exceed the established cut-off value (2.0- or 0.5-fold).

Conclusion

This study confirmed that exposure to As, Pb, and Mn can collectively cause neurotoxicity, and both TH and VMAT2 play an important role in the toxicological effects of mixed exposure to As, Pb, and Mn.

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Conflict of interest: None

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Ethics statement: Experiments were approved by the Institutional Animal Care and Use Committee of Keimyung University, Korea (approval No.: KM 2018-014). Experiments were conducted according to NIH guidelines for the care and use of laboratory animals.

References

1. Armijos RX, Weigel MM, Obeng-Gyasi E, Racines-Orbe M. Elevated blood lead and metal/metalloid levels and environmental exposure sources in urban Ecuadorian

school-age children and mothers. Int J Hyg Environ Health. 2021;235:113770.
2. Chen QY, Costa M. Arsenic: A global environmental challenge. Annu Rev Pharmacol Toxicol. 2021;61:47-63.
3. Amuno S, Rudko DA, Gallino D, Tuznik M, Shekh K, Kodzhahinchev V, et al. Altered neurotransmission and neuroimaging biomarkers of chronic arsenic poisoning in wild muskrats (Ondatra zibethicus) and red squirrels (Tamiasciurus hudsonicus) breeding near the City of Yellowknife, Northwest Territories (Canada). Sci Total Environ. 2020;707:135556.
4. Kim M, Seo S, Sung K, Kim K. Arsenic exposure in drinking water alters the dopamine system in the brains of C57BL/6 mice. Biol Trace Elem Res. 2014;162(1-3):175-80.
5. Malavika L, Mitra P, Goyal T, Sharma S, Purohit P, Sharma P. Association of blood lead level with neurobehavior and neurotransmitter expressions in Indian children. Toxicol Rep. 2021;8:971-6.
6. Ivleva I, Pestereva N, Zubov A, Karpenko M. Intranasal exposure of manganese induces neuroinflammation and disrupts dopamine metabolism in the striatum and hippocampus. Neurosci Lett. 2020;738:135344.
7. Berke JD. What does dopamine mean? Nat Neurosci. 2018;21(6):787-93.
8. Turac G, Duruksu G, Karaoz E. The effect of recombinant tyrosine hydroxylase expression on the neurogenic differentiation potency of mesenchymal stem cells. Neurospine. 2018;15(1):42-53.
9. Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, et al. The dopamine transporter: immunochemical characterization and localization in brain. J Neurosci. 1995;15(3 Pt 1):1714-23.
10. Mulvihill KG. Presynaptic regulation of dopamine release: Role of the DAT and VMAT2 transporters. Neurochem Int. 2019;122:94-105.
11. Caudle WM. Occupational metal exposure and Parkinsonism. Adv Neurobiol. 2017;18:143-58.
12. Srivastava P, Dhuriya YK, Gupta R, Shukla RK, Yadav RS, Dwivedi HN, et al. Protective effect of curcumin by modulating BDNF/DARPP32/CREB in arsenic-induced alterations in dopaminergic signaling in rat corpus striatum. Mol Neurobiol. 2018;55(1):445-61.
13. Tavakoli-Nezhad M, Barron AJ, Pitts DK. Postnatal inorganic lead exposure decreases the number of spontaneously active midbrain dopamine neurons in the rat. Neurotoxicology. 2001;22(2):259-69.
14. Sriram K, Lin GX, Jefferson AM, Roberts JR, Chapman RS, Chen BT, et al. Dopaminergic neurotoxicity following pulmonary exposure to manganese-containing welding fumes. Arch Toxicol. 2010;84(7):521-40.
15. Fortune T, Lurie DI. Chronic low-level lead exposure affects the monoaminergic system in the mouse superior olivary complex. J Comp Neurol. 2009;513(5):542-58.
16. Cobbina SJ, Chen Y, Zhou Z, Wu X, Feng W, Wang W, et al. Low concentration toxic metal mixture interactions: Effects on essential and non-essential metals in brain, liver, and kidneys of mice on sub-chronic exposure. Chemosphere. 2015;132:79-86.
17. Spurgeon DJ, Jones OA, Dorne JL, Svendsen C, Swain S, Stürzenbaum SR. Systems toxicology approaches for understanding the joint effects of environmental chemical mixtures. Sci Total Environ. 2010;408(18):3725-34.
18. Lister LJ, Svendsen C, Wright J, Hooper HL, Spurgeon DJ. Modeling the joint effects of a metal and a pesticide on reproduction and toxicokinetics in Lumbricid earthworms. Environ Int. 2011;37(4):663-70.